Electronic Supporting Information for:

A Minimalistic Catalytically-Active Cell Mimetic Made of a Supramolecular Hydrogel Encapsulated into a Polymersome

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Materials

PEG_{114}-b-PBG_{20} polymer was purchased from Polypeptide Therapeutic Solutions, S. L. and used without further purification.

Solvents were purchased from commercial suppliers and used without further purification.

All the solvents used were filtrated through a nylon membrane (pore size: 0.45 μm) before experiment was performed.

ImVal8 was synthesized as reported previously.[1]

Characterization Techniques

NMR Spectroscopy. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Agilent VNMR System 500 MHz at room temperature at a frequency of 500 and 125 MHz, respectively.

Optical Microscopy. Samples (10 μL) were applied directly onto sample plates and were collected directly. Optical images were acquired in air at room temperature, using a LEICA DMRME Optic Microscope with digital image 100-1000x.

Transmission Electron Microscopy (TEM). Samples (10 μL) were applied directly onto a 200 mesh carbon coated copper grids and were collected directly without staining. TEM images were recorded using a JEOL 1010 Transmission Electron Microscope.

Cryogenic Transmission Electron Microscopy (Cryo-TEM). Cryo-TEM samples were prepared from polymersomes or **ImVal8**-loaded polymersomes solutions. Glow discharge technique (15

sec, 7,2 V, using a Bal-Tec MED 020 Coating System) was applied over Lacey carbon-coated copper grids. Immediately, 4 μl of sample were put on the grid and vitrified using an automatic plunge freezer (Leica EM GP, Leica Microsystems Company, Wetzlar, Germany) at 10 ºC and 99% humidity. Excess sample was removed by blotting once 2.5 seconds with filter paper and plunged into liquid ethane at -180 ºC. After vitrification process, the grids were stored immersed in liquid nitrogen until use. The grids were mounted in a Gatan 626 cryo-holder (Gatan Company, California, USA) and examined using low dose software under a transmission electron microscope FEI Tecnai G2 Spirit BioTwin (ThermoFisher Scientific company, Oregon, USA). Pictures were taken with a digital camera Morada (EMSIS GmbH, Münster, Germany).

Cryogenic Scanning Electron Microscopy (Cryo-SEM). Cryo-SEM samples were loaded on the sample holder and cryo-fixed in slush nitrogen (-210 ºC) and then quickly transferred to the cryo-unit in the frozen state. Whole-polymersome samples (Figure 2A, B) were prepared on top of a nylon microfilter (0.45 μm pore), casted on top of a sample holder and, after freezing, directly sublimed at -90 ºC for 5 min. in the vacuum SEM chamber. Samples to be fractured (Figure 2C, D) were prepared by placing a drop between two pin holders and breaking them apart in the frozen state at the vacuum SEM chamber prior to sublimation at -90 ºC during 15 min. Cryo-SEM images were obtained with a Karl Zeiss Ultra 55 field emission scanning electron microscope with a cryoSEM PP3010T Quorum Technologies module under an accelerating voltage of 1.5-2 kV. Using this technique, the frozen samples were observed after Pt-sputtering for 30 seconds at 5 mA, while being maintained at -140 ºC.

Confocal laser scanning fluorescence microscopy (CLSFM). Confocal laser scanning fluorescence micrographs were obtained with a Leica TCS SP8 microscope equipped with a Diode 405, Argon (458, 488, 514), DPSS 561 and HeNe 633 lasers to perform the excitation. Microscope has three

dry objective lens 5x, 10x and 20x, two oil immersion objective lens 40x and 63x, and three fluorescence filters DAPI, FITC, RHOD, and the data files were processed using LAS X software. Using confocal laser scanning fluorescence microscopy, the samples were imaged via incorporating nile red (**NR**, 25 μM), rhodamine 6G (**R6G**, 10.4 mM), 8-hydroxypyrene-1,3,6-trisulfonate (**PyOH**, 0.6 mM) or 8- acetoxypyrene-1,3,6-trisulfonate (**PyOAc**, 0.6 mM) during the preparation process.

UV-visible spectroscopy. UV-visible absorption measurements were recorded using a JASCO FP-8300 spectrometer.

Fluorescence spectroscopy. Fluorescence measurements were collected using a JASCO FP-8300 spectrometer.

Flow cytometry. Flow cytometry measurements were collected using a BD Accury C6 equipped with two lasers blue (488 nm) and red (640 nm), two scattered light detectors (forward and side) and four fluorescence emitted light detectors (533 / 30 nm (FITC / GFP), 585 / 40 nm (PE / PI), >670 nm (PerCP, PerCP-CyTM5.5, PE-Cy7), 675 / 25 nm (ACP), 610 / 20 nm, 630 / 30 nm, 780 / 60 nm).

Zeta Potential measurements. The Zeta Potential measurements were performed at 25 °C using a Malvern Zetasizer Nano ZS instrument equipped with a 532 nm laser at a fixed scattering angle of 173°. Zeta potential value was measured for each conjugate with n > 4 measurements.

Protocols and results

Determination of critical vesiculation concentration (CVC). The CVC is the lowest concentration of polymer to form vesicles/polymersomes in water. To determine this value, a stock solution of

pyrene (3 mg) was prepared in 25 mL acetone.[2] 10 μL of this stock solution was added to 9 small vials, which were kept open overnight to evaporate the acetone. After that, the polymersome solution was diluted with MilliQ-water at different concentrations (1, 2, 4, 8, 32, 64, 125 and 250 $\mu g \cdot mL^{-1}$) and 1 mL of each concentration was added to the different vials and stirred overnight. Fluorescence intensities were recorded by exciting the different solutions at 334 nm, using a 5 nm slit width for excitation and a 5 nm slit width for emission. The fluorescence emission spectra were collected between 350 and 500 nm. The intensity of I_1 (371.7 nm) was plotted against the log of the concentration of each vial sample. The CVC was obtained by calculating the intersection of the two linear fits.

Preparation of giant polymersomes. PEG_{114}-b-PBG_{20} polymersomes were prepared using the emulsion solvent evaporation method (or oil-in-water emulsion solvent evaporation method).[3] Briefly, the di-block copolymer was dissolved in $CHCl_3$ (10 $mg \cdot mL^{-1}$) and the solution was added to a 10 mL of MilliQ-water, to reach a final concentration of 0.1 $mg \cdot mL^{-1}$. The obtained dispersion was vortexed for 1 min and then was magnetically stirred for 24 h at room temperature. After that, solution was centrifuged by VivaSpin 2 (3000 MWCO), and the pH was changed by dialysis (BIOTECH RC MEMBRANE, MWCO 20 kDa) against MilliQ-water for 2 days, changing the medium every 3 h during the day. For some specific experiments, solution was centrifuged for 10 min at 6000 rpm and polymersomes were separated and re-dispersed in 800 μL of MilliQ-water or 30 mM TRIS-HCl pH=7 buffer.

*Self-assembly studies of **ImVal8***. In order to load **ImVal8** into the polymersomes, it is essential to know its Critical Aggregation Concentration (CAC). This can be determined by fluorescence using

Nile Red (**NR**). For that, the peptide was dissolved in deionized water at different concentrations (typically above and below its minimum gel concentration (cgc)) and allowed to equilibrate for 2 h. To 800 μL of each solution, 1 μL of 1 mg·mL^{-1} **NR** solution in acetone was added. The different solutions were analyzed by fluorescence exciting at 550 nm and collecting the fluorescence emission spectra between 575 and 740 nm. The maximum emission wavelength value was plotted against the log of each concentration, and the CAC was calculated using the GraphPad Prism 4 software, obtaining a value of 0.15 mM.

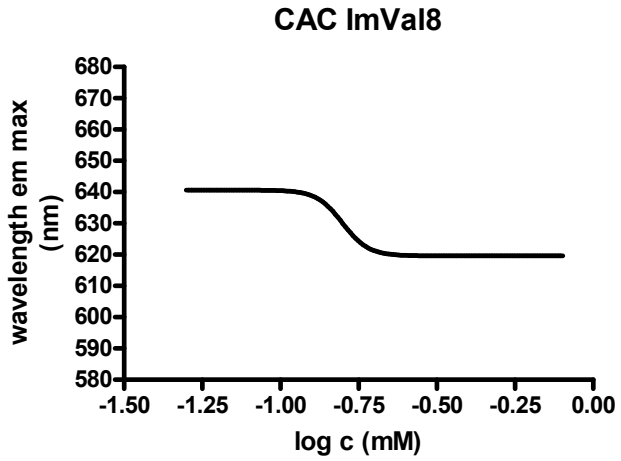

Figure ESI1. Determination of CAC of **ImVal8** using NR as a probe.

*Preparation of **ImVal8**-loaded giant polymersomes.* Polymersomes loaded with ImVal8 were prepared using the emulsion solvent evaporation method described above. In brief, PEG_{114}-b-PBG_{20} copolymer was dissolved in $CHCl_3$ (10 mg·mL^{-1}) and were added to a 10 mL solution of 0.15 mM of **ImVal8** at pH 3.5, to reach a final concentration of 0.1 mg·mL^{-1}. Note that at this acidic pH, **ImVal8** is completely dissolved in the solution. The polymersome solution was vortexed for 1 min and then magnetically stirred for 24 h at room temperature. After that, the solution was centrifuged by VivaSpin 2 (3000 MWCO), and the pH was changed by dialysis (BIOTECH RC

MEMBRANE, MWCO 20 kDa) against MilliQ-water for 2 days, changing the medium every 3 h during the day. It is worth mentioning that the **ImVal8** aggregates at neutral and basic pHs. For some specific experiments, solution was centrifuged for 10 min at 6000 rpm and polymersomes were separated and re-dispersed in 800 µL of MilliQ-water or 30 mM TRIS-HCl pH=7 buffer.

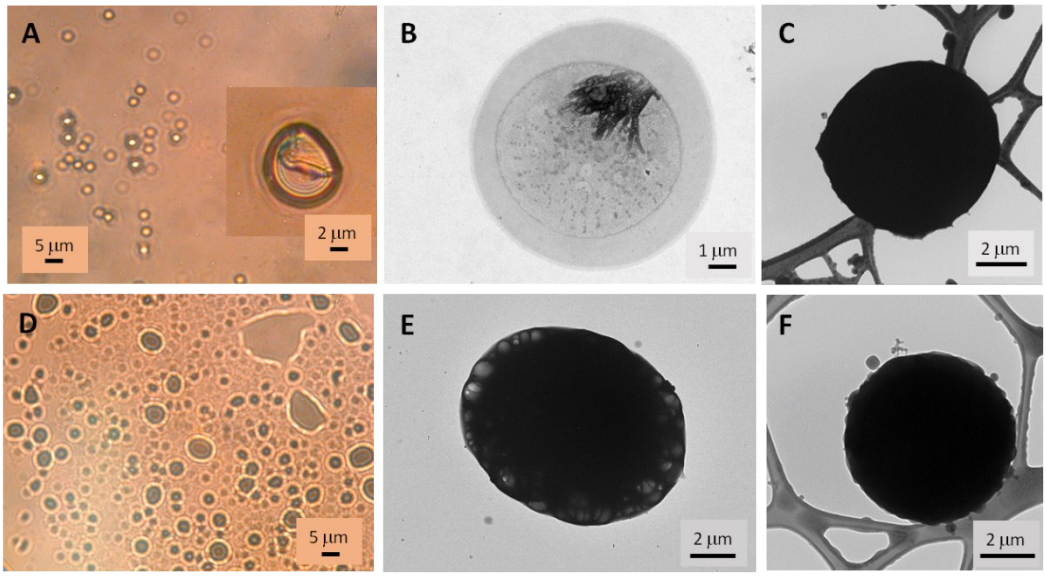

Figure ESI2. **PEG_{114}-b-PBG_{20} empty polymersomes** (A) Optical microscopy (B) TEM (C) Cryo-TEM. **PEG_{114}-b-PBG_{20} polymersomes loaded with ImVal8** (D) Optical microscopy (E) TEM (F) Cryo-TEM.

Hydrophobic dye loading. The hydrophobic dye Nile Red loading was performed according to Thayumanavan *et al*.[4] A stock solution of Nile Red at 2.5 mM in acetone was prepared. 1 µL of this stock solution was added to 100 µL of polymersome or **ImVal8**-loaded polymersomesolution and stirred for 24 h at room temperature. The vial was kept open to evaporate the acetone from the solution.

Hydrophilic dye loading. The water-soluble **R6G** dye loading was performed according to Thayumanavan *et al*.[4] The R6G dye (1 mg) was dissolved in 100 µL MeOH, and the organic solvent

was evaporated under rotary evaporation to form a thin film. 200 μL of polymersome or ImVal8-Loaded polymersome solution were added to this film with constant stirring and sonicated for 30 minutes. The mixture solution was magnetically stirred for 2 h at room temperature and after that, the solution was dialysed against MilliQ-water for 2 days, changing the medium every 3 h during the day. Pyranine dyes loading could not be performed according to Thayumanavan *et al.*[4] In that case, PEG_{114}-b-PBG_{20} copolymer was dissolved in $CHCl_3$ (10 $mg \cdot mL^{-1}$) and added to a of 0.15 mM solution of **ImVal8** at pH 3.5 with **PyOH** or **PyOAc** 0.6 mM, to reach a polymer final concentration of 0.1 $mg \cdot mL^{-1}$. Note that at this acidic pH, the **ImVal8** is completely dissolved. The polymersome solution was vortexed for 1 min and then magnetically stirred for 24 h at room temperature. After, the solution was ultrafiltrated with a Vivaspin 2 centrifugal concentrator, and the concentrated volume (200 μL) was dissolved with 1 mL of TRIS-HCl 30 mM pH=7 buffer solution. It is worth mentioning that the **ImVal8** aggregates at neutral and basic pHs. For some experiments, ultracentifugation and pH modification by buffer solution were changed by 10 min of centrifugation at 6000 rpm and dialysis against MilliQ-water for 24h, changing the medium every 3 h during the day.

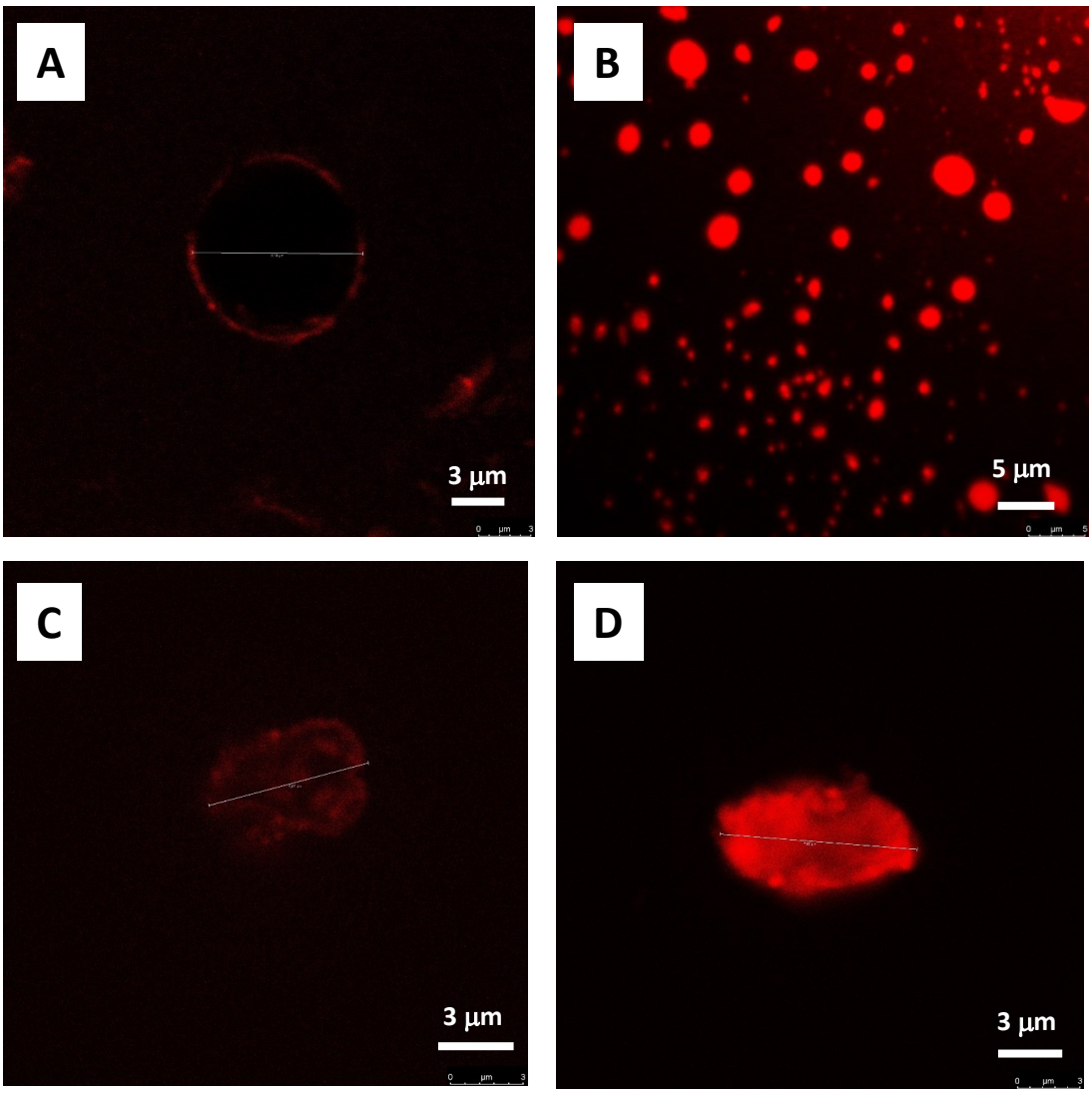

Fig. ESI3. CLSFM images of **NR** (A, B) and **R6G** (C, D) incorporated into empty (A, C) and **ImVal8**-filled (B, D) polymersomes.

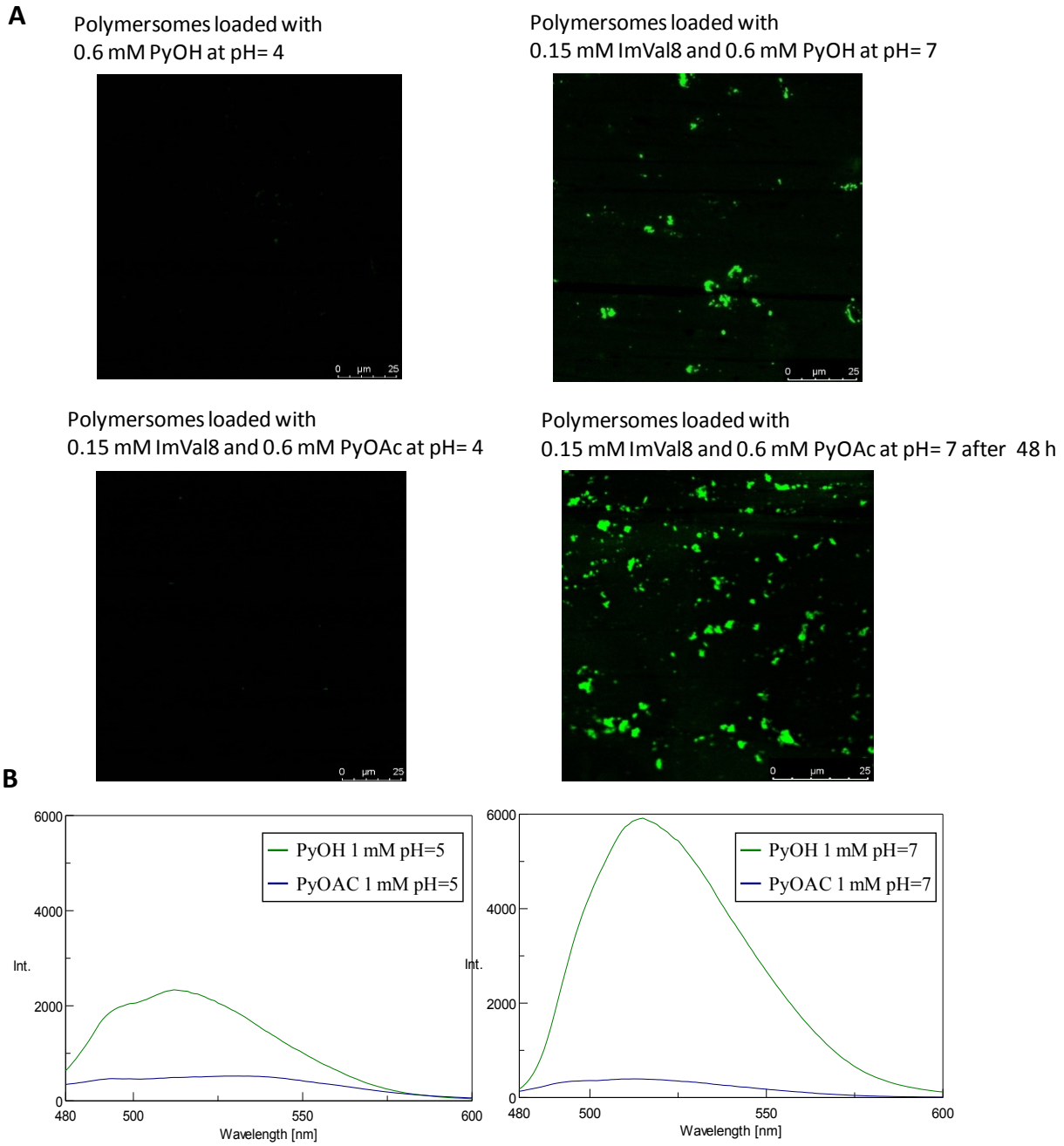

Figure ESI4. (A) CLSFM images of polymersomes loaded with **PyOH** or **PyOAc** at acidic and neutral pHs. (B) Fluorescence spectra of **PyOH** and **PyOAc** at acidic and neutral pHs.

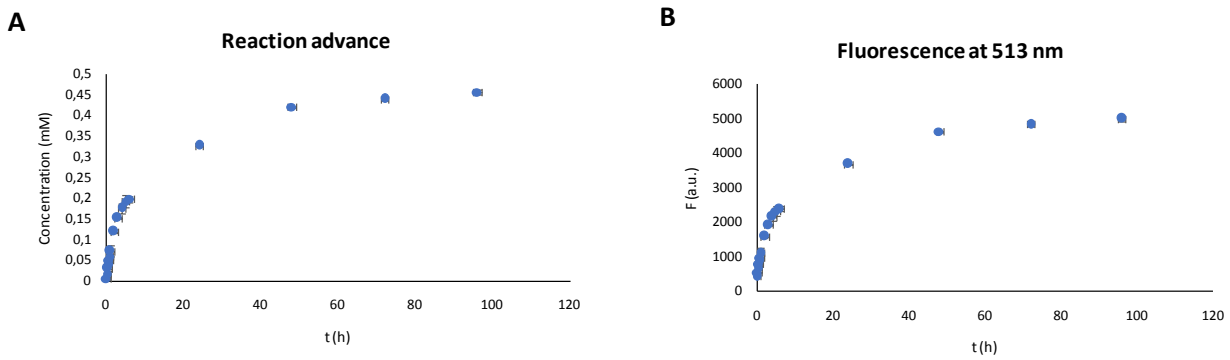

Figure ESI5. **Fluorescence analysis of 0.15 mM ImVal8 catalysis and 0.6 mM PyOAc (50% of catalyst) (n=3).** (A) Time profile of the percentage of reaction advance. (B) Time profile of the total sample fluorescence detected by fluorimetry at 513 nm.

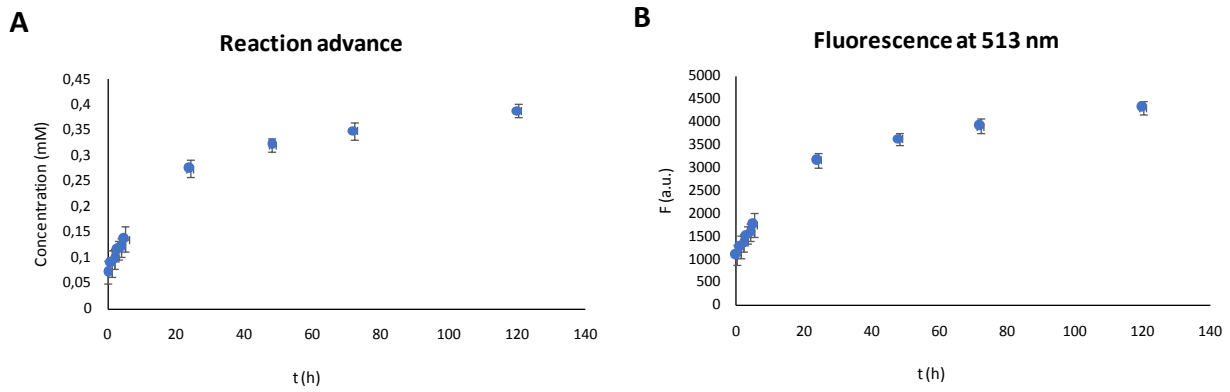

Figure ESI6. **Fluorescence analysis of catalytic jelly polymersomes loaded with 0.15 mM ImVal8 catalysis and 0.6 mM PyOAc (50% of catalyst) (n=3).** (A) Time profile of the percentage of reaction advance. (B) Time profile of the total sample fluorescence detected by fluorimetry at 513 nm.

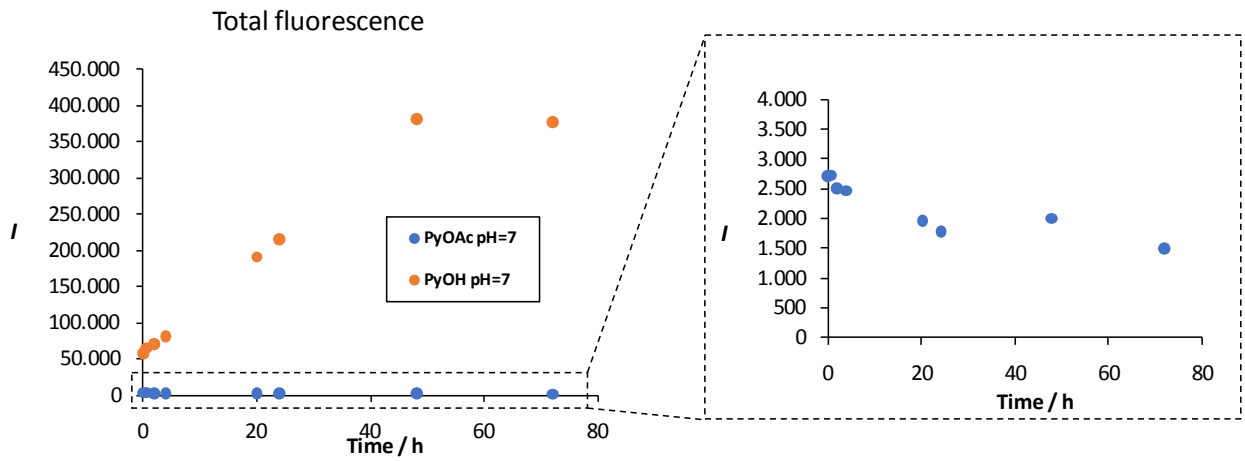

Figure ESI7. **Flow cytometry analysis of catalytic jelly polymersomes loaded with 0.15 mM ImVal8 and 0.6 mM PyOAc.** Time profile of the total sample fluorescence detected by flow cytometry.

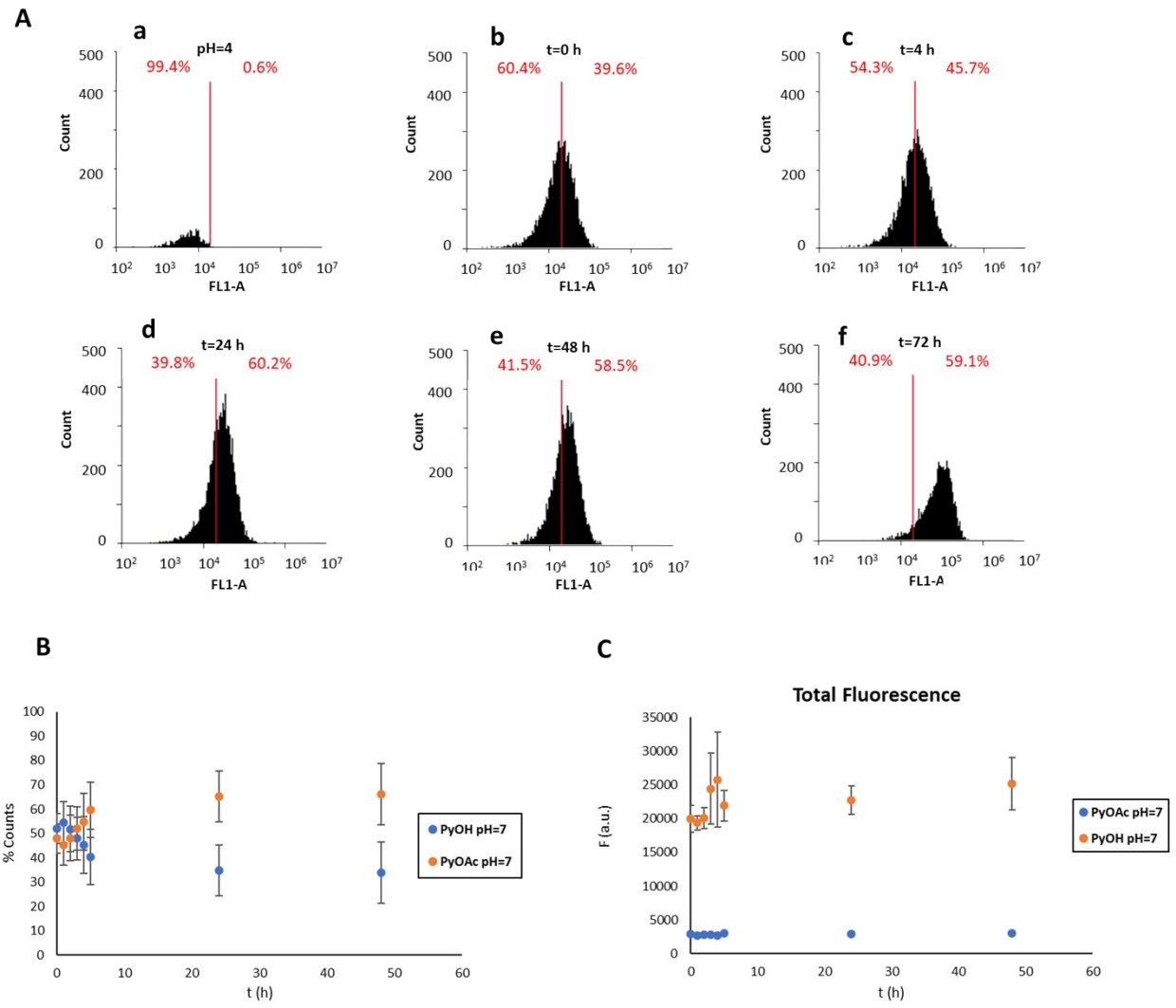

Figure ESI8. **Flow cytometry analysis of catalytic jelly polymersomes loaded with 0.4 mM ImVal8 and 1.6 mM PyOAc (50% of catalyst) (n=3)** (A) Particle fluorescence intensity increase with time. **a**. pH 4, **b-f** pH 7 at time intervals (**b**: t=0, **c**: t=4h, **d**: t=24h, **e**: t=48h, **f**: t=72h). Vertical lines point to the product threshold of fluorescence. (B) Time profile of the percentage of particles loaded with substrate and product. (C) Time profile of the total sample fluorescence detected by flow cytometry (total number of particles = 10.000).

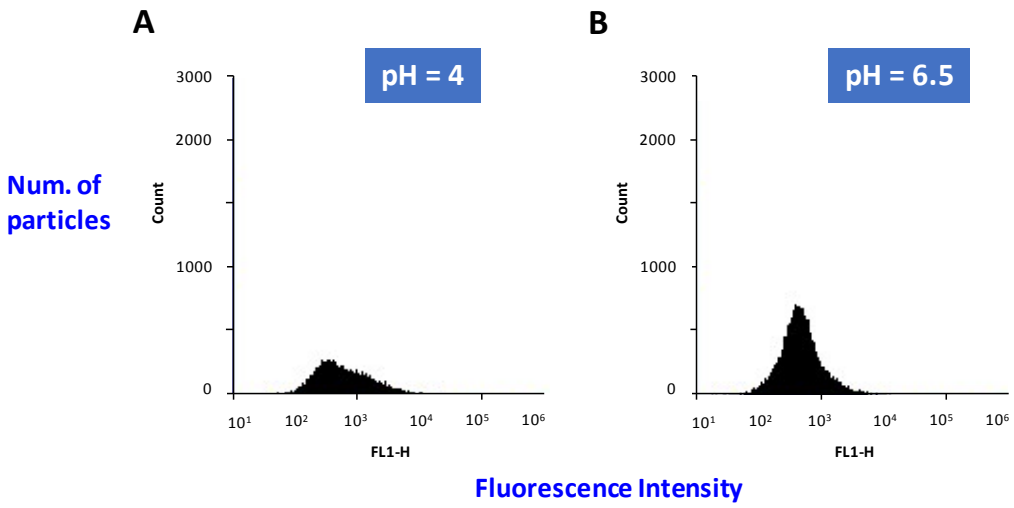

Figure ESI9. **Flow cytometry analysis of empty polymersomes loaded with0.6 mM PyOAc** (A) Particle fluorescence intensity at pH 4. (B) Particle fluorescence intensity at pH 6.5.

References

[1] N. Singh, M. P. Conte, R. V. Ulijn, J. F. Miravet, and B. Escuder, *Chem. Commun*., 2015, **51**, 13213-13216.

[2] (a) W. Chen and J. Du, *Sci. Rep.* 2013, **3**, 2162. (b) M. Wang, C. Zhou, J. Chen, Y. Xiao, and J. Du, *Bioconjugate Chem.* 2015, **26**, 725-734. (c) Y. Hong, Y. Xi, J. Zhang, D. Wang, H. Zhang, N. Yan, S. He, and J. Du, *J. Mater. Chem. B* 2018, **6**, 6311-6321.

[3] Y. Lee, J.-B. Chang, H. Kee Kim and T. Gwan Park, *Macromol. Res.*, 2006, **14**, 359-364.

[4] M. R. Molla, P. Rangadurai, L. Antony, S. Swaminathan, J. J. de Pablo and S. Thayumanavan, *Nat. Chem.* 2018, **10**, 659-666.